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Assessment of variability in temperature tolerance and antagonistic activity among *Trichoderma* isolates for biological control applications

Chithra Vinod, S.S. Veena*, J. Sreekumar, S. Karthikeyan and M.L. Jeeva

ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram 695 017, Kerala, India

Abstract

Trichoderma is a well-known genus of fungi, widely recognized for its role in biocontrol within agricultural systems. These fungi are commonly found in the soil and root zones of plants, where they contribute to enhance plant health and control diseases caused by soil-borne pathogens. The use of *Trichoderma*, either alone or in combination with organic amendments, is recommended for managing major diseases in tropical tuber crops. These include tuber rot and stem and root rot in cassava, anthracnose in yam, collar rot and postharvest rot in elephant foot yam, and taro leaf blight. Understanding the environmental conditions, particularly temperature tolerance, is crucial to optimize the effectiveness of *Trichoderma* across different climates. The present study examined the variability in both antagonistic potential and temperature tolerance of 97 *Trichoderma* isolates collected from various regions across the country. The antagonistic potential was assessed against *Sclerotium rolfsii*, pathogen that causes collar rot in elephant foot yam. The isolates demonstrated a wide range of mycelial growth inhibition, from 66.11% to 100%, with 17 isolates achieving complete inhibition of *S. rolfsii*. All isolates were able to grow within a temperature range of 15°C to 35°C, with optimal growth observed at 30°C, followed by 35°C. Two isolates were capable of thriving at temperatures as high as 40°C indicating their valuable use in regions with extreme climatic conditions. This variation in antagonistic potential and temperature tolerance among the isolates is valuable for selecting and developing effective isolates for specific crops, pathogens, and regions.

Keywords: *Trichoderma*, Temperature tolerance, Tuber crops, Antagonism, *Sclerotium rolfsii*

Introduction

Plant diseases play a direct role in destroying natural resources in agriculture and are said to be a major cause of reducing the annual level of food production in the world, which, depending on the source, is estimated at the level of 10-40% (Ghorbanpour, 2018). An important part of sustainable agriculture practices is the effective management of plant diseases. Chemical pesticides induce resistance in pathogens, making them challenging to control after years of continuous application (Hawkins et al., 2018).The damage caused by the use of pesticides in the environment and human and animal health has been widely documented (Jepson et al. 2020; Richardson et al., 2019).

The mindset of consumers is changing to organic forms of production. With green agricultural development, people are urgently seeking safe, effective, and environmentally friendly plant disease control measures. Biocontrol agents (BCA) play an important role in agriculture, considered as one of the safe and effective strategy to control harmful

* Corresponding author
E-mail: veena.ss@icar.gov.in; Ph: +91 9497536500

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organisms in plants through beneficial organisms and their products and to increase plant food production globally, diminishing the necessity for chemical pesticides and fertilizers and offering a more sustainable and eco-friendly option. (Widmer, 2019; Besset-Manzoni, 2019; Harman, 2000).

Several species of *Trichoderma* are known to be versatile, opportunistic plant symbionts and are are among the most used and studied microorganisms as biocontrol agent owing to its range of biocontrol traits, such as parasitism, antibiosis, secondary metabolites (SM) production, and plant defense system induction (Guzmán-Guzmán et al. 2023). It can not only prevent diseases but also promotes plant growth, improves nutrient utilization efficiency and enhances plant resistance. *Trichoderma* mainly exists in the soil, air, plant surface, and other ecological environments (Haouhach et al., 2020; Zhang et al., 2021; Wang et al., 2022) and is a safe, low-cost, effective, eco-friendly bio-control agent for different crop species (Yao et al., 2023). The development of successful inoculation systems and effective delivery methods has enabled *Trichoderma* to stimulate plant growth and provide biocontrol against plant diseases (Abdullah et al., 2021).

One of the major problems associated with the use of beneficial microbes in agriculture is the variability of their effects on target organisms, which can be affected by environmental conditions. The application of *Trichoderma* as an antagonist is owing to their ability to survive under different adverse environmental conditions and *Trichoderma* by themselves are not immune to abiotic stresses such as moisture deficiency, higher temperature, etc., which tend to cause morphological, physiological, biochemical, and molecular changes and adversely affect the beneficial results of these bioagents (Haque et al. 2020; Poosapati et al. 2021).The use of species of *Trichoderma* is somewhat undermined by their variable level of bio-control activity, which is influenced by environmental conditions. Understanding the source of this variability is essential for its profitable and wide use in plant protection (Di Lelio, 2021).

Studies indicate that high atmospheric temperatures combined with low humidity are key environmental factors affecting the growth, sporulation and biocontrol efficacy of *Trichoderma* species. Temperature variations can influence the metabolic activities of these fungi, thereby impacting their interactions with pathogens and plants, and is a major factor contributing to the inconsistent performance of *Trichoderma* under field conditions (Poosapati et al., 2021). Optimal temperature ranges for *Trichoderma* growth and activity are species-specific and can significantly impact their performance in field conditions. The identification of thermo tolerant strains of this genus would be relevant, apart from the economic significance entailed (Poosapati et al., 2014). Therefore, studying the temperature response of different *Trichoderma* strains is essential for developing effective biocontrol strategies and for selecting strains that are best suited to specific climatic conditions.

The tropical tuber crops are susceptible to many pathogens in field as well as in storage which lead to significant economic loss. Fungal pathogens, such as *Fusarium* spp*, S. rolfsii*, *Colletotrichum gloeosporioides* and oomycete. *Phytophthora colocasiae* are among the most important ones, causing diseases such as tuber rot and stem and root rot in cassava, collar rot in elephant foot yam, anthracnose in yam and taro leaf blight (Jeeva et al. 2020; Prakash et al., 2023). Application of *Trichoderma* alone or in combination with chemicals is being practiced for the management of various fungal diseases of tropical tuber crops (Baby et al., 2022). Among the field diseases of elephant foot yam, collar rot caused by *S. rolfsii* Sacc. is the most destructive and common disease prevalent in all the elephant foot yam growing areas (Kumar et al., 2017; Aswathy et. al., 2019). Organic growers have limited options for managing diseases of tuber crops since most of the effective fungicides, fumigants and seed treatments are synthetic, toxic and potentially polluting (Veena et al., 2013). Application of *Trichoderma* spp is recommended as the eco-friendly strategy to combat the crop loss. However, all *Trichoderma* isolates may not perform equally against specific soil borne pathogens as *Trichoderma* antagonists have different mechanisms of pathogen recognition (Linet et al., 2018). In this study, ninety seven *Trichoderma* isolates have been evaluated for their ability to grow at different temperatures and capacity to inhibit the mycelial growth of *S. rolfsii.*

Statistical analysis

The data on mycelial growth of pathogens at different concentrations of various fungicides and mycelial growth at different temperatures were statistically analyzed. Mean separation was determined according to Duncan's multiple range test ($p<0.05$).

Materials and Methods

Microbial cultures

Ninety-seven isolates of *Trichoderma* obtained from the tuber crop ecosystem across various parts of India (Kerala, Karnataka, Andhra Pradesh, and Odisha) and maintained in the microbial repository at ICAR-CTCRI were used for this study. Similarly, *S. rolfsii* isolated from an elephant foot yam plant (variety 'Gajendra') showing typical symptoms of collar rot and maintained in the ICAR-CTCRI microbial repository was used. All cultures were sub-cultured on Potato Dextrose Agar (PDA) medium and incubated at 28±2°C. Cultures were periodically transferred and fresh sub-cultures were used for the study.

Evaluation of *Trichoderma* Isolates for antifungal activity against *Sclerotium rolfsii*

The differential antagonistic potential of 97*Trichoderma* isolates was evaluated against *Sclerotium rolfsii* using the dual culture method as described by Skidmore and Dickinson (1976). To assess inhibitory action, mycelial discs (5 mm in diameter) from actively growing cultures of each *Trichoderma* isolate were co-inoculated with *S. rolfsii* on a single PDA plate. Each *Trichoderma* disc was placed 3 cm from the periphery of the plate. Similarly, mycelial discs from actively growing *S. rolfsii* cultures were positioned directly opposite the *Trichoderma* discs, maintaining a 3 cm distance between the two. Each isolate was tested in triplicate, with *S. rolfsii* inoculated alone on PDA plates as the control. The radial mycelial growth of *S. rolfsii* was recorded every 24 hours. On day 3, the control plates showed complete coverage (90 mm) by *S. rolfsii*. The radial growth of *S. rolfsii* in the presence of each *Trichoderma* isolate was measured, and the percentage inhibition was calculated relative to the control, using the following formula:

$$1 = \frac{C\text{-}T}{C} \times 100 \quad \text{... Eqn. (1)}$$

Where, I = percent inhibition; C = radial growth of pathogen (in mm) alone in the control plate; T = radial growth of pathogen (in mm) in the presence of *Trichoderma* isolates (Edington et al., 1971).

Effect of temperature on mycelial growth of *Trichoderma*

The effect of temperature on the mycelial growth of *Trichoderma* isolates was assessed by incubating cultures at six different temperatures: 15, 20, 25, 30, 35, and 40°C. The experiment was conducted in a BOD (Biochemical Oxygen Demand) incubator to maintain precise environmental conditions. Mycelial discs, each measuring 5 mm in diameter, were excised from the actively growing edge of each *Trichoderma* isolate culture and placed centrally on Potato Dextrose Agar (PDA) plates. The plates were observed, and the radial mycelial growth was recorded at intervals of 24, 48, and 72 hours of post-incubation to evaluate the growth rate across different temperatures. Each isolate was tested with three replicates to ensure the reliability of results, and the average mycelial growth at each temperature was calculated to determine the optimal temperature range for mycelial expansion.

Results and Discussion

Screening of *Trichoderma* isolates against *S. rolfsii*

All the isolates tested expressed their potential to arrest the mycelial growth of *S. rolfsii*, the pathogen responsible for collar rot disease in elephant foot yam. However, the isolates varied in their antagonistic action towards *S .rolfsii* (Table 1). *Trichoderma* isolates, T2, T21, T26, T27, T30, T34, T39, T45, T46, T53, T59, T73, T78, T79, T89, T91, and T93 showed 100% mycelial growth inhibition consistently across all time points (24 h, 48 h, and 72 h). These isolates demonstrated strong potential as reliable biocontrol agents against *S. rolfsii*. Few isolates, T14, T20, T22, T23, and T24 showed a gradual increase in inhibition percentage from 24 hours to 72 hours, indicating a cumulative suppressive effect on the pathogen over time. Isolates, T11 and T63 exhibited low inhibition percentages across all time points, with values below 70%. These isolates may not be suitable for individual use in controlling *S. rolfsii*, though they might still offer benefits when combined with other bio-agents. In control plates, mycelium of *S. rolfsii* completely covered the medium by 72 h (3 days after inoculation (DAI)) and all the 97 isolates showed >65% mycelial growth inhibition against *S. rolfsii* on that day. At 72 h of inoculation, majority of the isolates were with 80-90% inhibition (67%) and only 1.34% of the isolates expressed <80% inhibition (Fig. 1).

Table 1. Inhibition of mycelial growth of *S. rolfsii* by *Trichoderma* isolates

Isolate number	Mycelial growth inhibition (%) after		
	24 h	48 h	72 h
T1	$30.95^{fghijklm}$	67.59^{ijk}	$80.56^{ghijklmn}$
T2	100.00^{a}	100.00^{a}	100.00^{a}
T3	$35.71^{defghijk}$	69.44^{ghijk}	78.33^{klmno}
T4	42.86^{defgh}	72.22^{defghi}	78.88^{jklmno}
T5	69.05^{bc}	74.07^{defghi}	$84.44^{defghijkl}$
T6	$26.19^{hijklmn}$	70.37^{fghij}	$82.22^{defghijklmn}$
T7	$30.95^{fghijklm}$	73.15^{defghi}	$81.11^{fghijklmn}$
T8	$30.95^{fghijklm}$	71.30^{efghij}	75.56^{nop}
T9	47.62^{def}	79.63^{def}	$85.55^{defghij}$
T10	$30.95^{fghijklm}$	68.52^{hijk}	$80.56^{ghijklmn}$
T11	21.43^{jklmn}	52.78^{l}	66.11^{gq}
T12	16.67^{lmn}	67.59^{ijk}	$80.56^{ghijklmn}$
T13	19.05^{klmn}	67.59^{ijk}	78.88^{jklmno}
T14	50.00^{de}	78.70^{defg}	87.22^{cdefg}
T15	$38.09^{defghij}$	75.00^{defghi}	$85.00^{defghijk}$
T16	40.48^{defghi}	73.15^{defghi}	$83.33^{defghijklm}$
T17	$35.71^{defghijk}$	74.07^{defghi}	$84.44^{defghijkl}$
T18	$35.71^{defghijk}$	72.22^{defghi}	$82.22^{defghijklmn}$
T19	$38.09^{defghij}$	74.07^{defghi}	$83.33^{defghijklm}$
T20	45.23^{defg}	76.85^{defghi}	86.11^{defghi}
T21	100.00^{a}	100.00^{a}	100.00^{a}
T22	47.61^{def}	76.851^{defghi}	$85.55^{defghij}$
T23	47.61^{def}	76.85^{defghi}	86.11^{defghi}
T24	100.00^{a}	80.56^{cde}	87.78^{bcdef}

T25	45.23^{defg}	75.93^{defghi}	85.55^{defghij}
T26	100.00^{a}	100.00^{a}	100.00^{a}
T27	100.00^{a}	100.00^{a}	100.00^{a}
T28	52.38^{cd}	77.78^{defgh}	85.00^{defghijk}
T29	50.00^{de}	79.63 ^{def}	86.67^{defgh}
T30	100.00^{a}	100.00^{a}	100.00^{a}
T31	52.38^{cd}	78.70^{defg}	86.11^{defghi}
T32	52.38^{cd}	78.70^{defg}	87.22^{cdefg}
T33	100.00^{a}	90.74^{ab}	93.89^{abc}
T34	100.00^{a}	100.00^{a}	100.00^{a}
T35	45.23^{defg}	77.78^{defgh}	85.55^{defghij}
T36	40.47^{defghi}	76.85^{defghi}	85.55^{defghij}
T37	50.00^{de}	77.78^{defgh}	86.11^{defghi}
T38	28.57^{ghijklmn}	69.44^{ghijk}	81.67^{efghilklmn}
T39	100.00^{a}	100.00^{a}	100.00^{a}
T40	45.23^{defg}	75.93^{defghi}	84.44^{defghijkl}
T41	100.00^{a}	74.07^{defghi}	79.44^{ijklmno}
T42	45.24^{defg}	76.85^{defghi}	86.11^{defghi}
T43	100.00^{a}	89.81^{bc}	93.89^{abc}
T44	42.85^{defgh}	73.15^{defghi}	82.22^{defghijklmn}
T45	100.00^{a}	100.00^{a}	100.00^{a}
T46	100.00^{a}	100.00^{a}	100.00^{a}
T47	50.00^{de}	77.78^{defgh}	86.67^{defgh}
T48	40.47^{defghi}	75.00^{defghi}	85.00^{defghijk}
T49	40.47^{defghi}	74.07^{defghi}	81.67^{efghilklmn}
T50	100.00^{a}	90.74^{ab}	88.88^{bcd}
T51	47.61^{def}	77.77^{defgh}	86.66^{defgh}
T52	50.00^{de}	73.14^{defghi}	83.33^{defghijklm}
T53	100.00^{a}	100.00^{a}	100.00^{a}
T54	45.23^{defg}	77.77^{defgh}	86.11^{defghi}
T55	47.61^{abcde}	77.77^{defgh}	86.11^{defghi}
T56	100.00^{a}	90.74^{ab}	94.44^{ab}
T57	50.00^{de}	77.77^{defgh}	86.11^{defghi}
T58	42.85^{defgh}	73.14^{defghi}	82.22^{defghijklmn}
T59	100.00^{a}	100.00^{a}	100.00^{a}
T60	33.33^{efghijkl}	74.07^{defghi}	83.33^{defghijklm}
T61	52.38^{cd}	78.70^{defg}	86.66^{defgh}
T62	33.33^{efghijkl}	71.29^{efghij}	83.33^{defghijklm}
T63	11.90 ^{n}	56.48 ^{l}	68.88^{pq}
T64	28.57^{ghijklmn}	71.29^{efghij}	81.67^{efghilklmn}
T65	40.47^{defghi}	74.07^{defghi}	84.44^{defghijkl}
T66	23.80^{ijklmn}	60.18 ^{kl}	73.33^{op}
T67	71.42^{b}	75.00^{defghi}	84.44^{defghijkl}
T68	30.95^{fghijklm}	71.29^{efghij}	81.11^{fghijklmn}
T69	35.71^{defghijk}	70.37^{fghij}	81.67^{efghilklmn}
T70	14.28^{mn}	62.03^{jkl}	76.66^{mno}
T71	100.00^{a}	81.48^{bcd}	88.88^{bcd}
T72	100.00^{a}	77.77^{defgh}	85.55^{defghij}
T73	100.00^{a}	100.00^{a}	100.00^{a}
T74	35.71^{defghijk}	68.51^{hijk}	81.11^{fghijklmn}
T75	38.09^{defghij}	69.44^{ghijk}	81.11^{fghijklmn}
T76	33.33^{efghijkl}	69.44^{ghijk}	77.77^{lmno}
T77	35.71^{defghijk}	71.29^{efghij}	81.67^{efghilklmn}
T78	100.00^{a}	100.00^{a}	100.00^{a}
T79	100.00^{a}	100.00^{a}	100.00^{a}
T80	47.61^{def}	74.07^{defghi}	82.22^{defghijklmn}
T81	45.23^{defg}	75.00^{defghi}	85.00^{defghijk}
T82	100.00^{a}	100.00^{a}	94.44^{ab}
T83	30.95^{fghijklm}	67.59^{ijk}	78.88^{jklmno}
T84	45.23^{defg}	73.14^{defghi}	80.00^{hijklmno}
T85	40.47^{defghi}	75.92^{defghi}	85.55^{defghij}
T86	100.00^{a}	76.85^{defghi}	85.55^{defghij}
T87	45.23^{defg}	75.00^{defghi}	85.00^{defghijk}
T88	50.00^{de}	77.77^{defgh}	85.55^{defghij}
T89	100.00^{a}	100.00^{a}	100.00^{a}
T90	100.00^{a}	90.74^{ab}	87.77^{bcdef}
T91	100.00^{a}	100.00^{a}	100.00^{a}
T92	76.19^{b}	79.62 ^{def}	87.77^{bcdef}
T93	100.00^{a}	100.00^{a}	100.00^{a}
T94	76.19^{b}	81.48^{bcd}	88.33^{bcde}
T95	100.00^{a}	80.55^{cde}	88.33^{bcde}
T96	52.38^{cd}	78.70^{defg}	87.22^{cdefg}
T97	52.39^{cd}	81.48^{bcd}	87.77^{bcdef}

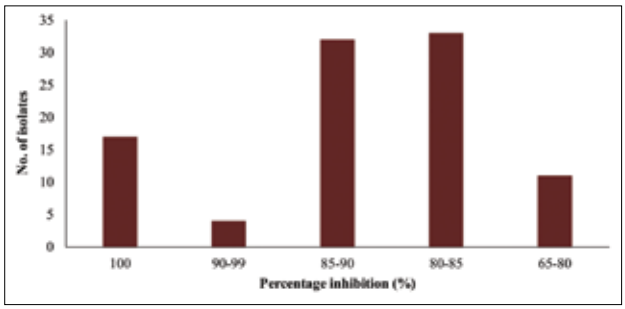

Fig.1. Distribution of number of isolates based on antagonistic activity against *S. rolfsii*

In recent decades, chemical pesticides have been widely used to protect crops from fungal pathogens, but their safety and environmental impacts raise serious concerns (Ghorbanpour, 2018). Overuse can lead to pesticide resistance in pathogens, soil and water contamination, and harm to beneficial insects, soil microbiomes, and ecosystems (Alizadeh et al., 2020). Sustainable approaches, such as Integrated Pest Management (IPM) and organic farming, help mitigate these issues (Grasswitz, 2019). One promising strategy is using biological

control agents (BCAs), which employ microorganisms, their metabolites, or natural products to control plant pathogens (Thambugala et al., 2020). Bioagents are crucial for managing plant diseases (Li et al., 2013). Among them, *Trichoderma* is particularly effective as a biocontrol agent against economically significant soil-borne pathogens. Abundantly found in soil and root ecosystems, *Trichoderma* serves as a potent antagonist to fungal pathogens affecting plants (Kushwaha et al., 2014; Shahid and Srivastava, 2014). *Trichoderma*, a dominant component of various soil ecosystem mycobiomes, are characterized by the ability to colonize plant roots. The growing interest in the application of *Trichoderma* results from their direct and indirect biocontrol potential against a wide range of soil phytopathogens (Ty´skiewicz et al., 2022).

Biological control, particularly using *Trichoderma* species, has emerged as a promising approach for sustainable management of *S. rolfsii* - induced diseases (Adhikari et al., 2022). Application of *Trichoderma* is recommended to manage collar rot in elephant foot yam. The differential antagonistic potential of 43 *Trichoderma* isolates were assessed by adopting three *in vitro* screening methods viz., direct confrontation, antibiosis test based on production of diffusible inhibitory metabolites and production of volatile compounds. In dual culture method, percentage inhibition of mycelial growth of pathogen varied from 9.44% to 82.32% (Linet et al., 2018). Comparable results have been obtained by various researchers across different crops. Motlagh et al. (2022) studied the effectiveness of *T. viride* and *T. virens* as biocontrol agents against *S. rolfsii*, the causative agent of peanut stem white rot, finding that *T. virens* achieved the highest suppression of *S. rolfsii* mycelial growth in dual culture (90.98%). Similarly, *T. viride* showed 78.61% inhibition, followed by *T. harzianum* at 75.28% against *S. rolfsii*, which causes collar rot in chickpea under in vitro conditions (Meena et al. 2023). The rate of inhibition was fastest with *T. harzianum* (63.60%), followed by *T. virens* (51.5%), while *T. viride* showed the least inhibition (50.85%) after 72 hours (Kushwaha et al. 2018). The results of the study indicated the presence of highly efficient isolates in the collection, with the majority (86 isolates) showing more than 80% inhibition. Further studies on these isolates could advance the development of alternative management options to control the pathogen *S. rolfsii*.

Effect of temperature on growth of *Trichoderma* isolates

All ninety-seven isolates were able to grow within a temperature range of 15-35°C (Table 2). The growth rate varied significantly depending on the isolate and the temperature. The growth rate ranged from 0.43 cm day^{-1} (isolate T91 at 15°C) to 3.8 cm day^{-1} (isolate T89 at 35°C). The mean mycelial growth of the isolates at 30°C was significantly higher than at all other temperatures, followed by 35°C (Fig. 2). The lowest growth rate was observed at 15°C, which was significantly lower than the mycelial growth rates recorded at all other temperatures. Fifteen isolates -T3, T19, T20, T52, T59, T63, T66, T73, T79, T81, T87, T92, T94, T95, and T96 exhibited a growth rate difference of less than 0.5 cm day^{-1} across temperatures of 20°C, 25°C, 30°C and 35°C. These isolates may be suitable for regions with significant temperature variations across different seasons.

Table 2. Mycelial growth rate of *Trichoderma* isolates at different temperatures

Isolate No.	Mycelial growth rate (cm day^{-1}) of various isolates at				
	15°C	20°C	25°C	30°C	35°C
T1	0.889	2.083	2.183	2.700	2.567
T2	0.856	2.217	2.700	2.833	2.533
T3	0.800	2.167	2.300	2.500	2.200
T4	0.944	1.917	2.300	3.033	2.100
T5	0.856	2.067	2.133	2.783	2.267
T6	0.856	2.050	2.367	2.800	2.367
T7	1.333	1.850	2.650	3.200	3.167
T8	1.111	2.133	3.083	3.267	2.783
T9	0.822	2.150	2.683	3.200	2.300
T10	0.878	2.133	2.283	3.000	2.633
T11	0.967	2.217	2.617	2.900	2.367
T12	0.867	2.117	2.450	2.833	1.900
T13	0.856	2.133	2.433	2.783	2.367
T14	0.833	2.233	2.283	2.800	2.000
T15	0.844	2.200	3.067	2.900	2.600
T16	0.844	2.167	3.483	3.100	2.733
T17	0.989	2.200	2.300	2.800	2.100
T18	0.900	2.183	3.400	2.700	2.400
T19	0.889	2.000	2.450	2.533	2.500
T20	0.889	2.100	2.067	2.400	2.267
T21	0.967	2.150	2.650	3.000	2.700
T22	0.911	2.267	2.733	3.100	3.000
T23	0.900	2.017	3.733	2.933	2.983
T24	0.889	2.067	2.500	3.200	2.500
T25	0.933	1.867	2.367	3.067	3.000
T26	0.856	2.067	2.317	3.200	3.400
T27	0.911	1.950	2.117	2.633	2.400
T28	1.500	2.250	2.650	3.233	3.200
T29	0.833	1.667	1.783	2.733	2.367
T30	0.822	1.300	1.650	2.267	1.567
T31	0.856	1.517	2.083	2.667	2.500
T32	0.833	1.633	3.017	2.667	2.567

T33	0.978	1.650	2.733	2.533	2.433
T34	0.556	1.483	1.767	2.367	2.400
T35	0.822	1.733	1.883	2.783	2.600
T36	1.367	2.10	2.533	2.900	3.200
T37	0.922	1.800	2.600	2.467	2.333
T38	0.789	1.783	2.500	2.667	1.533
T39	1.167	2.000	2.983	2.983	2.600
T40	0.833	1.683	2.750	2.467	2.167
T41	1.156	1.050	3.683	2.800	2.783
T42	0.933	2.017	2.350	2.983	2.800
T43	0.856	1.867	2.950	3.100	2.983
T44	1.033	1.733	2.333	3.600	3.667
T45	1.067	1.850	3.700	3.100	2.400
T46	1.000	1.517	1.783	3.400	3.200
T47	0.833	1.767	2.333	2.700	2.400
T48	0.989	2.000	2.217	2.600	2.400
T49	0.867	1.850	3.017	3.033	3.300
T50	0.933	1.350	2.183	2.000	1.400
T51	1.100	1.983	2.300	3.067	3.033
T52	1.333	2.517	2.650	2.983	3.000
T53	1.167	1.900	2.650	3.333	3.267
T54	0.922	2.033	2.817	3.467	2.867
T55	0.900	2.000	3.133	3.100	2.500
T56	1.078	2.150	2.850	3.400	2.700
T57	1.053	2.050	2.400	2.700	2.467
T58	0.567	1.800	2.650	3.033	2.533
T59	1.167	2.350	2.517	2.800	2.700
T60	1.000	2.133	2.650	3.000	2.733
T61	1.067	2.000	2.217	2.733	2.333
T62	1.000	2.283	2.650	3.233	3.133
T63	1.000	1.950	2.250	2.333	2.067
T64	1.100	2.350	2.633	3.267	2.833
T65	0.800	1.800	2.300	3.267	3.267
T66	1.033	2.283	2.650	2.500	2.283
T67	1.300	2.000	3.083	2.867	2.067
T68	1.067	2.100	2.300	3.200	3.133
T69	1.200	2.083	2.250	3.067	3.200
T70	1.200	2.000	2.383	2.867	2.700
T71	0.900	2.167	2.450	2.167	1.600
T72	1.367	2.250	2.850	2.567	2.000
T73	1.033	2.650	2.783	2.783	2.367
T74	0.767	2.217	2.650	3.033	2.983
T75	1.367	2.650	3.517	3.200	2.983
T76	1.233	2.400	3.183	3.167	2.833
T77	1.233	2.333	2.650	3.300	3.300
T78	1.333	2.317	2.650	3.367	3.267
T79	1.100	2.350	2.533	2.400	2.033
T80	1.000	2.217	2.650	2.467	1.967
T81	0.833	2.467	2.650	2.900	2.783
T82	1.000	2.483	2.650	3.267	3.167
T83	1.000	2.400	2.533	3.133	2.983
T84	1.000	1.967	2.650	2.983	3.467
T85	0.867	2.417	2.650	3.033	2.667
T86	1.000	2.033	2.350	2.733	2.500
T87	1.033	2.450	2.650	2.467	2.167
T88	1.067	2.017	2.150	3.333	3.567
T89	1.067	1.917	2.150	3.400	3.800
T90	0.500	1.967	2.050	2.983	3.067
T91	0.433	2.483	2.650	3.233	3.167
T92	1.067	2.350	2.467	2.433	2.067
T93	0.700	1.967	2.050	3.267	3.233
T94	1.067	2.583	2.650	2.400	2.067
T95	1.100	2.650	3.000	2.533	2.500
T96	0.900	2.517	2.650	2.983	2.733
T97	1.000	2.483	2.650	3.300	3.200

CD values	
Treatment mean comparison	0.1026
Temperature mean comparison	0.0233
Interaction mean comparison	0.2296

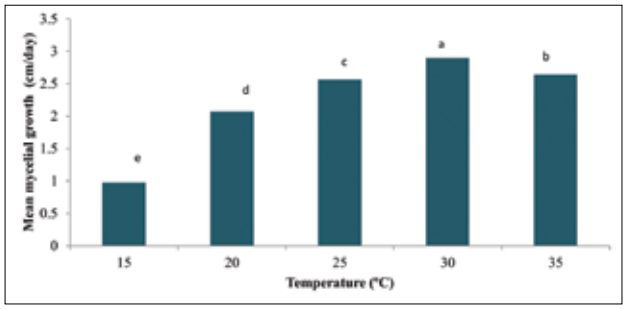

Fig. 2. Mean mycelial growth rate of isolates at 15ºC, 20ºC, 25ºC, 30ºC and 35ºC based on statistical analysis

At 40°C, only two isolates (isolates numbers T41 and T65) could grow. The growth pattern of the isolates at different time intervals is shown in Fig. 3.

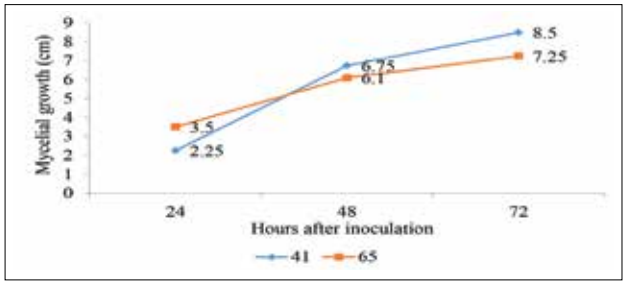

Fig. 3. Mycelial growth in isolates T41 and T65 after 24, 48 and 72 hours of incubation

Trichoderma isolates that thrive at high temperatures, such as 40ºC, hold significant potential for agricultural systems in tropical and arid regions where elevated temperatures may limit the effectiveness of conventional biocontrol agents. The isolates demonstrated 79.44% and 84.4% mycelial growth inhibition against *S. rolfsii*. By maintaining their antagonistic activity and effective colonization at elevated temperatures, these isolates ensure consistent biocontrol efficacy. Furthermore, they can be incorporated into formulations for improved shelf stability and broader applicability, making them a crucial resource for sustainable agriculture in warmer climates.

The selection of an appropriate *Trichoderma* isolate is influenced by various factors, including the intended use, ecological adaptability to different environments, availability of water and nutrients, climatic conditions, and the specific crops involved, all of which can affect their field efficacy (Hjeljord and Tronsmo, 1998). Several studies have shown that temperature plays a crucial role in spore germination, hyphal growth, and colonization, which in turn affects the competitive and antagonistic capabilities of *Trichoderma* (Kredics et al., 2003). The available literature suggests that an optimal temperature range of 25-30°C is ideal for mycelial growth. Both 25°C and 30°C have been identified as the best temperatures for the mass production of *Trichoderma* (Ghazanfar et al., 2018). Species such as *T. harzianum*, *T. viride*, *T. asperellum*, *T. koningii*, *T. atroviride*, and *T. longibrachiatum* were able to produce sufficient biomass at various temperatures, including 20°C, 25°C, 30°C, and 35°C, with optimal growth observed between 25°C and 30°C. No significant difference in growth was noted between 25°C and 30°C (Singh et al., 2014). While the maximum radial growth of *Trichoderma* isolates occurred at 25°C, the highest dry weight was recorded at 30°C (Haque et al., 2020).

Contreras-Cornejo et al. (2016) affirm that *Trichoderma* generally requires a temperature range of 25-30°C for optimal growth. However, certain species, such as *T. pseudokoningii*, are more temperature-tolerant and can thrive at higher temperatures, while many other species fail to grow above 28°C (Mukherjee et al., 2012). Research on *Trichoderma* consortia cultivation at varying temperatures has shown that the optimal growth temperature is around 25°C, where colonies achieve maximum diameter, biomass accumulation, sporulation, and conidia production (Shumenova et al., 2024). Moreover, *Trichoderma* treated tomato plants exposed to 25°C and 20°C temperatures exhibited significant differences in fungal biological performance. To effectively tackle the challenges posed by local environmental conditions and the extreme climatic shifts due to global warming, it is crucial to carefully select the most suitable *Trichoderma* isolates for field applications (Di Lelio, 2021). Most studies indicate that the ideal temperature for the mycelial growth of *Trichoderma* is 25-30°C. However, the isolates used in this study preferred temperatures ranging from 30°C to 35°C for optimal growth.

Conclusion

The study highlights the high variability among the ninety-seven *Trichoderma* isolates obtained from the rhizosphere of tropical tuber crops across different regions. These isolates demonstrated significant antagonistic potential, with most showing over 80% mycelial growth inhibition against *S. rolfsii*. This suggests that *Trichoderma* can be a highly effective biocontrol agent for managing soil-borne pathogens in tropical agriculture. The study also identified both temperature-stable and thermo tolerant isolates, which are capable of thriving at higher temperatures. These isolates are particularly valuable for use in regions with extreme climatic conditions or for crops grown during hot seasons, where high temperatures can hinder the performance of conventional biocontrol agents. Future studies should focus on scaling the use of thermo tolerant isolates and exploring their synergistic interactions with other biocontrol agents to improve field efficacy.

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